

WHAT IS CLAIMED IS:

1. A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:
- 5                   subjecting pine cells to *Agrobacterium* infection for *Agrobacterium* transformation;
- minimizing damage to cells subsequent to *Agrobacterium* infection;
- rapidly selecting transformed cells;
- culturing said transformed cells to produce transgenic somatic embryos; and
- 10                   germinating said transgenic somatic embryos to produce transgenic plants.
2. The method of claim 1, wherein said damage to cells is minimized by:
- (a) resuspending cells having been subjected to transformation in a liquid wash medium;
- (b) agitating said cells in said liquid wash medium;
- (c) recovering washed, transformed cells with minimal damage.
3. The method of claim 2, wherein pine cells are plated onto a support membrane prior to subjecting to *Agrobacterium* transformation.
4. The method of claim 1, wherein said damage to cells is minimized by:
- (a) plating pine cells on a support membrane;
- (b) rinsing said cells using a liquid wash medium;
- (c) recovering washed, transformed cells with minimal damage.
- 25                   5. The method of claim 4, wherein pine cells are plated onto a support membrane prior to subjecting to *Agrobacterium* transformation.
6. The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to subjecting to *Agrobacterium* transformation.
- 30                   7. The method of claim 4, wherein steps (b) and (c) are repeated until *Agrobacterium* contamination is no longer detectable.

Sub  
A1

09973088.10100

8. The method of claim 7, wherein said steps are repeated between 2 and 10 times.

Sub A2 5  
9. ~~The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium without interfering with subsequent growth of the plant cells.~~

10. ~~The method of claim 9, wherein each wash is carried out for between half an hour to overnight in duration.~~

10  
11. The method of claim 4, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Sub A2 10  
12. ~~The method of claim 1, wherein said rapid selection is performed by  
culturing cells which have been subjected to transformation on a support  
membrane placed over a gel medium;  
contacting said cells with a selection agent; and  
selecting transformed cells.~~

13. The method of claim 12, wherein said selection agent is contained in said gel medium.

14. The method of claim 12, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is placed on said gel medium.

25  
15. ~~The method of claim 14, wherein said layer is a thin film of liquid medium.~~

Sub A1  
16. ~~The method of claim 14, wherein said layer is a thin film of gelled medium.~~

30 17. The method of claim 14, wherein said layer is a filter paper with a liquid medium absorbed therein.

18. The method of claim 12, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

5 19. The method of claim 1 which further comprises the eradication of *Agrobacterium*.

sub  
A3 > 20. The method of claim 19, wherein said eradication is performed by:  
culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium and

10 recovering cells from which said *Agrobacterium* has been eradicated.

21. The method of claim 20, wherein said layer is a thin film of liquid medium.

5 22. The method of claim 20, wherein said layer is a thin film of gelled medium.

23. The method of claim 20, wherein said layer is a filter paper with a liquid medium absorbed therein.

20 24. The method of claim 20, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

25 25. A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

sub  
A6 > subjecting pine cells to *Agrobacterium* infection for *Agrobacterium* transformation;

eradicating *Agrobacterium*;

minimizing damage to cells during and subsequent to *Agrobacterium* eradication;

30 rapidly selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

09973088-101001

26. The method of claim 25, wherein said damage to cells is minimized by:
- (a) resuspending cells having been subjected to transformation in a liquid wash medium;
  - (b) agitating said cells in said liquid wash medium;
  - (c) recovering washed, transformed cells with minimal damage.
27. The method of claim 26, wherein pine cells are plated onto a support membrane prior to subjecting to *Agrobacterium* transformation.
28. The method of claim 26, wherein said rapid selection is performed by
- culturing cells which have been subjected to transformation on a support membrane placed over a gel medium;
  - contacting said cells with a selection agent; and
  - selecting transformed cells.
29. The method of claim 26, wherein said eradication is performed by:
- culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
  - recovering cells from which said *Agrobacterium* has been eradicated.
30. The method of claim 28, wherein said eradication is performed by:
- culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
  - recovering cells from which said *Agrobacterium* has been eradicated.
31. The method of claim 25, wherein said damage to cells is minimized by:
- (a) plating pine cells on a support membrane;
  - (b) rinsing said cells using a liquid wash medium;
  - (c) recovering washed, transformed cells with minimal damage.

5

10

15

20

25

30

Sub  
Ag  
Cont it

09973088-101001

32. The method of claim 31, wherein pine cells are plated onto a support membrane prior to subjecting to *Agrobacterium* transformation.
33. The method of claim 31, wherein pine cells are plated onto a support membrane subsequent to subjecting to *Agrobacterium* transformation.
34. The method of claim 31, wherein said rapid selection is performed by  
culturing cells which have been subjected to transformation on a support membrane placed over a gel medium;  
contacting said cells with a selection agent; and  
selecting transformed cells.
35. The method of claim 31, wherein said eradication is performed by:  
culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and  
recovering cells from which said *Agrobacterium* has been eradicated.
36. The method of claim 34, wherein said eradication is performed by:  
culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and  
recovering cells from which said *Agrobacterium* has been eradicated.
37. The method of claim 25, wherein said rapid selection is performed by  
culturing cells which have been subjected to transformation on a support membrane placed over a gel medium;  
contacting said cells with a selection agent; and  
selecting transformed cells.
38. The method of claim 25, wherein said eradication is performed by:

Sub  
As  
cont

09973088-10101

Sub  
A6  
could

culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

- 5
39. A method for minimizing damage to transformed cells of pine of the genus *Pinus* subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation which comprises:

- 10
- (a) washing transformed cells in a liquid wash medium;
  - (b) plating said cells on a support membrane;
  - (c) resuspending said cells in a liquid wash medium; and
  - (d) recovering washed transformed cells with minimal physical damage.

- 15
40. The method of claim 39, wherein (i) cells are plated onto a support membrane and (ii) said cells are transformed prior to step (a).

41. The method of claim 39, wherein steps (b) and (c) are repeated until *Agrobacterium* contamination is no longer detectable.

- 20
42. The method of claim 41, wherein said steps are repeated between 2 and 10 times.

- Sub  
A7
- 25
43. The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium without interfering with subsequent growth of the pine cells.

44. The method of claim 43, wherein each wash is carried out for between half an hour to overnight in duration.

- 30
45. The method of claim 39, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Sub A8 > 46. A method for pine cell tissue culture which comprises culturing pine cells on a support membrane placed over a gel medium.

5 47. The method of claim 46, wherein said support membrane is placed over a layer containing one or more culture components, said layer is positioned on said gel medium.

48. The method of claim 46, wherein said cells are plated onto said support membrane prior to culturing.

Sub A9 > 10 49. The method of claim 47, wherein said layer is a thin film of liquid medium.

50. The method of claim 47, wherein said layer is a filter paper with a liquid medium absorbed therein.

15 51. The method of claim 46, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

20 52. A method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells which have been subjected to transformation on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

25 53. The method of claim 52, wherein said selection agent is contained in said gel medium.

54. The method of claim 52, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is positioned on said gel medium

30 Sub A7 > 55. The method of claim 54, wherein said layer is a thin film of liquid medium.

09973088-101001

56. The method of claim 54, wherein said layer is a filter paper with a liquid medium absorbed therein.

57. The method of claim 52, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer.

58. A method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells on a support membrane over a layer containing an eradicator, said layer positioned in or over a gel medium; and

recovering cells from which said *Agrobacterium* contaminant has been eradicated.

59. The method of claim 58, wherein said layer is a thin film of liquid medium.

60. The method of claim 58, wherein said layer is a thin film of gelled medium.

61. The method of claim 58, wherein said layer is a filter paper with a liquid medium absorbed therein.

62. The method of claim 58, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

63. A transformed embryogenic culture prepared by the method of claim 39.

64. A transformed embryogenic culture prepared by the method of claim 52.

65. A transformed embryogenic culture prepared by the method of claim 58.

66. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 1.

Sub  
AR 10

09973088 101001

15

20

25

30



67. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 2.
- 5 68. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 4.
69. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 12.
- 10 70. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 19.
- 15 71. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 25.
72. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 26.
- 20 73. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 28.
74. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 29.
- 25 75. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 30.
76. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 31.
- 30 77. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 34.

09973088 101001

78. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 35.

5 79. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 36.

80. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 37.

10

81. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 38.

09073088-101001